REMARKS

Amendments to the Specification

Applicants have amended the specification to clarify the amino acid residues of the A chain of SLT-1. SEQ ID No.: 1 of the present application mistakenly refers to the A chain of SLT-1 as being 299 amino acids long. As the originally filed application makes clear, the A chain of SLT-1 is 293 and not 299 amino acids long, and corresponds to amino acid residues 7 to 299 of SEQ ID No.: 1.

The originally filed application provides ample support for the A chain of SLT-1 being 293 amino acids long; *see*, *e.g.*, at page 4, lines 21-22 and 28-30; page 6, lines 30-31; page 10, line 31 to page 11, line 2; and Figure 1. The originally filed application also refers to International Patent Publication No. WO 99/40185 in the "Background Of The Invention" section of the application (*see*, page 1, lines 29-31). WO 99/40185, in turn, describes that the A chain of SLT-1 is 293 amino acids long and an alignment of the 293 amino acid sequence disclosed in WO 99/40185 with the amino acid sequence of SEQ ID No.: 1 of the present application reveals that the 293 amino acid sequence of WO 99/40185 is identical to amino acid residues 7 to 299 of SEQ ID No.: 1. Thus, SEQ ID No.: 1 of the instant application includes an "extra" 6 residues and the A chain of SLT-1 spans from amino acid residue 7 to 299 of SEQ ID No: 1.

As further evidence that the A chain of SLT-1 is 293 amino acids long, the originally filed application contains multiple references to specific amino acid residues within the A chain and such residue numbering is based on a sequence that is 293 amino acids in length. For example, the originally filed application states that the protease-sensitive loop of the A chain of SLT-1 is defined by the only two cysteine residues in the

A chain at Cys 242 and Cys 261 (*see*, page 4, lines 24-26; page 6, line 31 to page 7, line 2; page 7, lines 13-14; and Figure 1). An inspection of SEQ ID No.: 1 of the present application, however, shows the same two unique cysteine amino acid residues numbered as Cys 248 and Cys 267 (i.e., differing by 6 amino acid residues). Similarly, the originally filed application states that certain amino acid residues are crucial for the catalytic activity of the A1 domain such as tyrosine 77, glutamic acid 167, arginine 170 and tryptophan 203 (*see*, page 4, lines 24-26; page 8, lines 2-6; and Figure 1). An inspection of SEQ ID No.: 1 reveals that the amino acid residue numbering for tyrosine 77, glutamic acid 167, arginine 170 and tryptophan 203 also differs by 6 amino acid residues (corresponding to tyrosine 83, glutamic acid 173, arginine 176 and tryptophan 209 of SEQ ID No.: 1, respectively).

Accordingly, for consistency and clarity, applicants have amended the specification to correctly number amino acid residues of the A chain of SLT-1 consistently with it having 293 amino acid residues corresponding to amino acid residues 7 to 299 of SEQ ID No.: 1.

Claim Amendments

Claims 1, 4, 5, 8, 9, 11, 12, 30 and 31 have been amended. Claims 8-35 have been withdrawn. Claim 6 has been canceled. This claim cancellation is specifically without waiver of applicants' right to seek claims to the cancelled subject matter in this application or in other applications claiming the benefit or priority of this application. Thus, claims 1-5 and 7-35 are pending in this application following entry of these amendments.

Applicants have amended claim 1 to specify that the toxic protein is an ABx toxic protein. Support for this amendment can be found throughout the application as originally filed, *e.g.*, at page 1, lines 8-19; and page 6, lines 13-22. Applicants have also amended claim 1 to improve its form by replacing the term "in" with the term "into" and specifying that the insert is introduced into the protease-sensitive loop or region of the A chain of the ABx toxic protein. Applicants have also amended withdrawn claims 8 and 9 in the same manner so that they remain commensurate in scope with the elected group.

Applicants have amended claim 4 to specify that the protein species comprise A1 domain sequences sufficient to retain catalytic activity and A2 domain sequences to permit introduction of the insert between amino acids 248 and 267, as defined with reference to SEQ ID No.: 1. Support for this amendment can be found throughout the application as originally filed, *e.g.*, at page 4, lines 21-23; and page 6, lines 25-27. Applicants have also amended claim 4 to improve its form by specifying that the insert be introduced between amino acids 248 and 267, as defined with reference to SEQ ID No.: 1. Applicants have also amended withdrawn claims 11 and 30 in the same manner so that they remain commensurate in scope with the elected group. Support for this amendment can be found throughout the application as originally filed, *e.g.*, at page 4, lines 24-26; page 6, line 31 to page 7, line 2; page 7, lines 13-14; and Figure 1.

Applicants have amended claim 5 to improve its form by specifying that the insert be introduced between amino acids 251 and 252, as defined with reference to SEQ ID No.: 1. Applicants have also amended withdrawn claims 12 and 31 in the same manner so that they remain commensurate in scope with the elected group. Support for

this amendment can be found throughout the application as originally filed, *e.g.*, at page 4, lines 24-26; page 6, line 31 to page 7, line 2; page 7, lines 13-14; and Figure 1.

None of the amendments introduces any new matter.

Sequence Compliance

The Examiner states that the application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825 for the reasons set forth in the Notice to Comply with Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures (hereinafter referred to as the "Notice", a copy of which is enclosed herewith). Specifically, the Notice states that Figures 2A and 2B, as well as specification page 4, line 29 and pages 16-17 (Tables 1-2) contain sequences without proper SEQ ID No identifiers. The Notice states that applicants must provide a substitute paper copy of the Sequence Listing as well as an amendment directing its entry into the specification as well as a substitute computer readable form (CRF) copy of the Sequence Listing. The Notice also states that applicants must provide a statement that the content of the substitute paper and computer readable copies of the Sequence Listing are the same and include no new matter as required by 37 C.F.R. §§ 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825(d).

Accordingly, applicants submit herewith a substitute CRF of the Sequence Listing that is in compliance with 37 C.F.R. §§ 1.821 – 1.825. The substitute Sequence Listing provides SEQ ID No identifiers for the sequences mentioned at Figures 2A and 2B, as well as at specification page 4, line 29 and pages 16-17 (Tables 1-2). The substitute Sequence Listing is submitted herewith in ".txt" format electronically via EFS-Web without a paper copy. Applicants also submit herewith a Statement Under 37 C.F.R.

§§ 1.821-1.825 that the amended CRF copy of the substitute Sequence Listing does not include new matter. Applicants have also amended the specification to insert the required SEQ ID No identifiers. Applicants have also requested replacement of the earlier filed Sequence Listing in the application with the substitute Sequence Listing submitted herewith.

Drawings

The Examiner states that the drawings are objected to because Figures 2A and 2B contain sequences without proper SEQ ID No identifiers.

Applicants have amended Figures 2A and 2B to insert the proper SEQ ID No identifiers and have submitted herewith Replacement Drawings. Accordingly, applicants request that the Examiner withdraw this objection.

Specification

The Examiner states that the specification is objected to because Figure 1 is duplicated in the abstract. The Examiner also states that specification page 4, line 29 and pages 16-17 (Tables 1-2) are objected to because they contain sequences without proper SEQ ID No identifiers. The Examiner further states that clarification regarding SEQ ID No.: 1 is requested because, while the specification states that the A chain is 293 amino acids in length, SEQ ID No.: 1 is 299 amino acids in length.

Applicants have amended the specification by deleting Figure 1 from the abstract.

Applicants have also amended the specification to insert the required SEQ ID No identifiers for the sequences at specification page 4, line 28 to page 5, line 4 and pages 16-17 (Tables 1-2).

Finally, applicants clarify that the A chain of SLT-1 is 293 amino acids long and that it corresponds to amino acid residues 7 to 299 of SEQ ID No.: 1. As discussed above, the originally filed application provides ample support that the A chain of SLT-1 is 293 amino acids long and corresponds to amino acid residues 7 to 299 of SEQ ID No.: 1.

Accordingly, applicants request that the Examiner withdraw these objections.

THE REJECTIONS

35 U.S.C. § 112, second paragraph
Claim 6

The Examiner has rejected claim 6 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Specifically, the Examiner states that, although independent claim 1 requires "an A chain of a toxic protein in which an insert has been introduced," which implies that the insert is within the A chain of a toxic protein, dependent claim 6 states that the insert is introduced "before or after amino acids 1-239 of the Shiga-like toxin I A chain".

Without addressing or in any way acquiescing to the Examiner's claim interpretation set forth above and solely to advance prosecution of this application,

applicants have canceled claim 6, thus rendering this rejection moot as to this claim. Accordingly, applicants request that the Examiner withdraw this rejection.

Claims 4-6

The Examiner has rejected claims 4-6 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Specifically, the Examiner states that it is unclear whether the claimed invention requires the full-length sequence of SEQ ID No.: 1 with an insert at the designated positions or if only a portion of SEQ ID No.: 1 is required. Applicants traverse in view of the claim amendments made herein.

Applicants have canceled claim 6, thus rendering moot the rejection as to this claim.

With respect to the remaining claims, applicants submit that it is clear from the language of amended claims 4 and 5 that the full-length sequence of SEQ ID No.: 1 is not required. Amended claims 4 and 5 depend indirectly from claim 1, which recites a combinatorial protein library comprising a plurality of protein species, each protein species comprising an A chain of an ABx toxic protein having a protease-sensitive loop or region into which an insert has been introduced. Thus, both amended claims 4 and 5 require that the protein species comprise, *i.e.*, includes at least the A chain of an ABx toxic protein, as long as there is sufficient A1 domain sequences to retain catalytic (toxic) activity and sufficient A2 domain sequence to permit insertion of the sequence at one of the recited positions in A2 (*see* Figure 1). (*See*, page 4, lines 21-23; *see* also, page 6, lines 25-27). Accordingly, applicants request that the Examiner withdraw this rejection.

35 U.S.C. § 102(b)
Claims 1-3 and 6-7

The Examiner has rejected claims 1-3 and 6-7 under 35 U.S.C. § 102(b) over U.S. Patent 6,080,400 ("Williams"). Specifically, the Examiner states that "Williams et al. teach fusion proteins (*i.e.*, library) comprising Shiga-like toxin I A chain fused to a polyhistidine tag, MBP, flag or other tags, wherein the tags are added at the N-terminus of the A chain (*i.e.*, before residue 1 of SEQ ID NO:1) and SEQ ID NO:47 (*i.e.*, 95.6% identity with SEQ ID NO:1) which comprises an insert between residues 4 and 5 and another insert between residues 6 and 7." (Office Action, page 8) Applicants traverse in view of the claim amendments made herein.

Applicants have canceled claim 6, thus rendering moot the rejection as to this claim.

With respect to the remaining claims, applicants have amended claim 1 (and therefore, dependent claims 2-3 and 7) to more clearly define the claimed invention. Specifically, applicants have amended claim 1 to recite that the combinatorial protein library comprises a plurality of protein species, each protein species comprising an A chain of an ABx toxic protein having a protease-sensitive loop or region ***into*** which an insert has been introduced, wherein the insert is a polypeptide of varying amino acid sequence having a length of at least 2 amino acid residues. Applicants submit that the amendment clearly specifies that the insert is introduced ***into*** an A chain of an ABx toxic protein rather than at the ends. This feature is nowhere taught or suggested by Williams.

Furthermore, the Examiner has mischaracterized Williams as teaching a library of fusion proteins. Amended claim 1 (and therefore, dependent claims 2-3 and 7)

recites a ***combinatorial library*** comprising a plurality of protein species. Williams does not teach or suggest a combinatorial library comprising a plurality of any type of protein species, let alone the specific protein species comprising an A chain of an ABx toxic protein having a protease-sensitive loop or region ***into*** which an insert has been introduced, as recited by the claims as amended herein.

Williams discloses expression of the A and B subunits of Verotoxin (an ABx toxic protein), which can then assemble to produce conformationally and functionally active Verotoxin protein identical to native forms. Williams describes the fusion of "tags" to the N-terminal ends of Verotoxin to facilitate purification of each of the expressed A and B subunits by virtue of the known affinity of the tag to another binding partner. Thus, Williams' purification method requires *attaching to the ends* of A and B subunits *a preselected binding sequence* (tag) known to have high affinity to a corresponding *preselected binding partner* (*e.g.*, a polyhistidine tag that binds to metal ions) in order to physically separate tagged subunits from untagged components by means of the high binding affinity between the tag and binding partner sequences. In contrast, the instant claimed invention is directed to a combinatorial library of protein species comprising a toxic A chain of an ABx toxic protein into which an insert has been introduced to *create an artificial binding domain of unknown affinity*, different and distinct from the normal binding specificity conferred by the B chain(s) which normally associate with the A chain of the ABx toxic protein to confer cell surface binding. The instant invention relies on inserting into a non-catalytic portion of the A chain a *variable* (rather than a known) *sequence having unknown binding properties* – the claimed insert's ability to confer novel and unknown binding affinities to a target ligand being a distinguishing feature of the presently claimed invention. To that end, the instant claims recite that the insert is "a polypeptide of *varying* amino acid sequence having a length of

at least 2 amino acid residues" unlike the terminal sequences of Williams, which are defined and not variable sequences, selected for the very reason that they have a known sequence and high affinity with a known binding partner. Nowhere does Williams disclose or suggest making an A chain combinatorial library comprising inserts having unidentified binding affinities in order to screen those inserts for their ability to bind a toxic A chain to a target ligand (*e.g.*, a cell surface receptor or ligand).

For all the above reasons, applicants request that the Examiner withdraw the claim rejections based on Williams.

Claims 1-3 and 6-7

The Examiner has rejected claims 1-3 and 6-7 under 35 U.S.C. § 102(b) over WO 99/40185 ("Gariepy"). Specifically, the Examiner states that Gariepy teaches polypeptide libraries comprising Shiga-like toxin A chain (SEQ ID NO:1) fused to hexahistidine at the N-terminus (*i.e.*, before residue 1). Applicants traverse in view of the claim amendments made herein.

Applicants have canceled claim 6, thus rendering moot the rejection as to this claim.

With respect to the remaining claims, applicants have amended claim 1 (and therefore, dependent claims 2-3 and 7) to more clearly define the claimed invention. As discussed above, applicants have amended claim 1 to recite that the combinatorial protein library comprises a plurality of protein species, each protein species comprising an A chain of an ABx toxic protein having a protease-sensitive region or loop ***into*** which an insert has been introduced. This feature is nowhere taught or suggested by Gariepy.

Instead, Gariepy describes libraries of mutant toxins in which mutations are introduced into the binding domain of mutant toxins to alter the type of cells to which the toxic species are delivered. The binding domain of mutant toxins mutated in Gariepy resides within the B subunit and not the A subunit of the toxic protein, which is the catalytic portion of the protein responsible for its cytotoxic activity. Thus, Gariepy does not anticipate amended claim 1 (and therefore, dependent claims 2-3 and 7) because it fails to teach introducing an insert into the catalytic A subunit of a toxic protein. Accordingly, applicants request that the Examiner withdraw the claim rejections based on Gariepy.

Provisional Obviousness-Type Double Patenting
Claims 1-7

The Examiner has provisionally rejected claims 1-7 under the judicially-created doctrine of obviousness-type double patenting as being allegedly unpatentable over claims 129-145 of copending U.S. Patent Application 12/088,206 ("the '206 application").

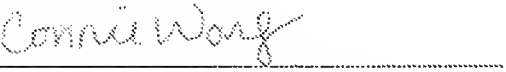
Applicants have canceled claim 6, thus rendering moot the rejection as to this claim.

Applicants request that this provisional rejection be held in abeyance until the claims of this application or the '206 application are found allowable. At that time, applicants will consider filing a Terminal Disclaimer as is appropriate and proper.

CONCLUSION

Applicants request favorable consideration of the application and early allowance of the elected claims.

Respectfully submitted,



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